Restriction Election

Applicants herein confirm the provisional election made on December 22, 2004 to prosecute Group I of the invention, claims 1-8 and 12-15. With regard to the species requirement, Applicants confirm the election of the following species: (i) $-CO-(CH_2)_x-(OCH_2CH_2)_mOR$ from Group A, (ii) monomethoxy polyethylene glycol groups from group B and (iii) a N-terminal residue from group C in satisfaction of the Examiner's species requirement.

REMARKS

With entry of this response, the claims pending are 1-2, 4-6, 8 and 12-15. Claims 3, 7, and 9-11 have been withdrawn by the Examiner as being drawn to a non-elected invention.

1. Claim rejections under 35 USC 103(a) Rejection.

The Examiner has rejected claims 1-2, 4-6, 8 and 12-13 under § 103(a) as being unpatentable over Namiki et al (EP 0816381 A1, published on 07/01/1998) in view of Date et al (Oncogene, 17:3045-3054, 1998) and Gaertner et al (Bioconjugate 7:38-42, 1996). This rejection is traversed.

The Examiner asserts that Namiki et al teach how to modify hepatocyte growth factor (HGF) by attaching a polyethylene glycol (PEG), and a pharmaceutical composition comprising the PEG modified HGF (page 12, line 37-40). However, Applicants note that Namiki only teaches the modification of HGF by attaching monoethoxy linear and branched PEG(s) at the N-terminus amino acids to improve the clearance and in vivo pharmacokinetics of HGF (page 2, line 49-57). The Examiner admits that Namiki does not teach NK4 nor the attachment of PEG with a molecular weight of 20 to 40 kDa.

The Examiner also asserts that since Gaertner allegedly discloses attaching PEG at amino terminus of proteins and that this suggests a PEG in size from 5 to 40 kDa should be attached to a protein (for an improved bioavailability) (page 44, first sentence of the 2nd paragraph of conclusion) at a single attachment point using oxime bond, that it would have been obvious to the person of ordinary skill in the art at the time the invention was made to attach PEG molecules in the range of molecular weight 5-40 kDa (as suggested by Gaertner) to the N-terminus amino acid of NK4 in order to increase clearance, improve in vivo pharmacokinetics as taught by Namiki with a HGF protein, and to prepare a pharmaceutical composition comprising the PEGylated protein.

According to the Examiner a person of ordinary skill in the art would have been motivated to attach NK4 as opposed to HGF with a reasonable level of success because Date et al show that NK4 molecule has antagonistic activities of HGF.

However, in contrast to the Examiner's assertions, the Nakimi reference teaches only how to modify HGF (hepatocyte growth factor) via "monoethoxy linear PEG" at the N-terminus location. As the Examiner admits, Nakimi does not teach the a) monomethoxy linear PEG addition, b) of molecular weight of 20-40kDa c) onto NK4 at the N-terminus location. The Gaertner (1996) reference discloses that while N-terminal serine or threonine are attractive for pegylation in certain proteins, it uses Il-8 (a cytokine) because "it has the appropriate N-terminal residue". Thus, the Gaertner reference merely teaches site-specific pegylation of Il-8, G-CSF and Il-1ra via different procedures to result in a more favorable site to pegylate. The site specific pegylation of G-CSF requires enzymatic cleavage/digestion of Met-Thr-Pro. The site specific pegylation of IL-1ra occurs via selective modification of the alpha amino group by metal-catalyzed transamination of Met-Arg-Pro, which has the drawback of exposing the protein to a metal - which results in side-reactions and lower yields. Importantly, however, Gaertner also admits that pegylation presents a big problem if the attachment sites are not precisely controlled, as this affects protein stability and functionality.

In other words, Gaertner et al. merely discloses a method for the PEGylation of a terminal serine and threonine amino acid. If no serine or threonine residue is available at the N-terminus of the protein, i) either additional steps are required to uncover a serine residue present within the last two amino acids of the protein (example with G-CSF) ii) or harsh oxidation conditions have to be employed (example with IL-1ra) yielding a plurality of side products and reducing the overall yield.

The PEGylation according to Gaertner et al. is thus accomplished by the formation of an oxime bond between an N-terminally introduced carbonyl group in the protein and an aminooxy functionalized PEG. This is in contrast to the formation of an

amide bond as reported in the current invention. Gaertner et al. also only teaches the attachment of linear PEG with a molecular weight of approx. 20 kDa.

Overall, Gaertner further admits the PEGylation presents a big problem if the attachment sites are not precisely controlled, as this affects protein stability and functionality.

Indeed, as disclosed by Applicants' specification, the Mehvar (2000) reference (which was published four years after Gaertner) states that conjugation of different polyethylene glycols to IL-8 and G-CSF as well as other interleukins results in the production of molecules with impaired properties (specification, paragraph 5). Furthermore, Francis et al. (1998) posits that "PEGylation of proteins is always based on trial and error and virtually all parameters of such a PEGylation can have a surprising and very profound effect on the functionality of the product" (specification, paragraph 6). Finally, as disclosed in our specification, Reddy (2000) states that each protein requires different optimization chemistry and therefore the influence of PEGylation cannot be predicted.

Accordingly, for the above reasons, Applicants respectfully submit that claims 1-2, 4-6, 8 and 12-13 are not anticipated nor made obvious by the cited references and are thus in condition for allowance.

2. The Examiner has rejected claims 14 and 15 under 35 USC 103(a) as being unpatentable over Namiki et al (EP 0816381 A1, published on 07/01/1998) in view of Date et al (Oncogene, 17:3045-3054, 1998) and Gaertner et al (Bioconjugate 7:38-42, 1996) and further in view of the Veronese reference. The Examiner asserts that since Veronese et al disclose making PEGylated proteins and purifying them to greater than 92% purity, Veronese would teach a higher purity PEGylated protein resulting in a better bioavailability and pharmacokinetics in vivo.

However, the addition of the Veronese reference the above analysis does not render Applicants' invention obvious. Veronese merely concerns the pegylation of HGRF and more particularly only site-specific pegylation (like Gaertner) at lysine or N-terminus tyrosine. It does not address pegylation at any other protein and, in fact, only claims a peg-hgf complex that does not contain a triazine group. Indeed, and in support of Applicants' invention, Veronese specifically admits that "site-specific pegylation remains a chemical challenge."

In contrast to the current invention, Veronese et al. reports only the PEGylation of HGRF and no general method because unique coupling conditions have to be used, which are optimized for hGRF. Further, in contrast to the current invention, the PEG is coupled to the protein via a norleucine or lyisine linker and not via a monomethoxy linker. Further in contrast to the current invention is a coupling of PEG to the terminal amino group only possible when all other lysine-amino-groups are protected. Still further in contrast to the current invention, Veronese et al. reports only the conjugation of a linear PEG of a molecular weight of 20 kDa. Accordingly, applicants respectfully suggest that claims 14-15 are not anticipated nor made obvious by Veronese and are thus in condition for allowance.

3. Conclusion

Summarizing, the current invention describes the PEGylation of an N-terminal amino acid of NK4 via amide bond formation between an N-terminal amino group and the a carboxyl group of a functionalized monomethoxy-PEG, with the molecular weight of the attached linear PEG is of between 20 kDa and 40 kDa. The cited references do not singly or in combination disclose or suggest a conjugate consisting of a NK4 molecule and a polyethylene glycol group having a molecular weight of about 20-40 kDa wherein polyethylene glycol group has: (i) the formula $-CO-(CH_2)_x-(OCH_2CH_2)_mOr$, (ii) is monomethoxy polyethylene glycol and (iii) forms amide group with the amino groups of N-terminal NK4 fragment.

For the reasons set forth above, Applicants respectfully submit that all claims 1-2, 4-6, 8 and 12-13, as herein presented, be hereby put into condition for allowance. Should the Examiner consider that outstanding issues remain unresolved, it is respectfully requested that the Examiner please telephone the undersigned attorney to discuss them.

Applicants believe that no further fee is required in connection the filing of this Amendment. If any additional fees are deemed necessary, authorization is given to charge the amount of any such fee to Deposit Account No. 08-2525.

Respectfully submitted,

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